bio-protocol Preprint

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Cell lysis

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An abbreviated version of this protocol was published in eLIFE in Apr 2020

Single-molecule functional anatomy of endogenous HER2-HER3 heterodimers

DOI: 10.7554/eLife.53934

Detailed protocol

1. Prepare lysis buffer base: 50mM HEPES-NaOH [pH 7.4], 150mM NaCl, 10% glycerol (v/v), 2% protease inhibitor cocktail (v/v) (SigmaAldrich P8340), 2% tyrosine phosphatase inhibitor cocktail (v/v; to preserve endogenous phosphorylation level) (SigmaAldrich P5726)
2. Dissolve mild detergent (digitonin or GDN at 1% w/v) into the lysis buffer base.
3. Add complete lysis buffer to the cell pellet (We used ~400ul of complete lysis buffer to lyse 10^7 of SKBR3 cell).
4. Using micro-pipet, resuspend cell pellet completely.
5. Incubate for 10 min at 4°C.
6. Repeat step 4~5, 3 times.
7. Centrifuge the mixture at 15000g, 4°C, for 10 min.
8. Collect supernatant.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Choi, B. (2020). Cell lysis. Bio-protocol Preprint. bio-protocol.org/prep306.
2. Choi, B., Cha, M., Eun, G. S., Lee, D. H., Lee, S., Ehsan, M., Chae, P. S., Heo, W. D., Park, Y. and Yoon, T.(2020). Single-molecule functional anatomy of endogenous HER2-HER3 heterodimers. eLIFE. DOI: 10.7554/eLife.53934